EXHIBIT D



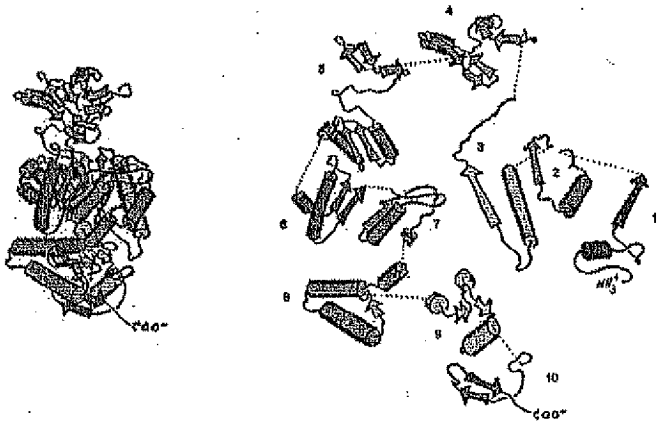

Figure 3. The Structure of PK and Intron Positions

PK is shown schematically as a protein with secondary and tertiary structure on the left. On the right, this structure is expanded at the positions of introns in the tertiary structure of the protein. α-Helix (barrels) and β-sheet (arrows) structures are illustrated. (This figure is reprinted with permission from Lonberg and Gilbert, 1985.)

That is, an exon corresponds to the minimal amount of information that is expressed as a discrete unit. This concept becomes particularly relevant when the trans-splicing process is considered (Agabian, 1990). In this case, exons transcribed from different loci (and, in many cases, different chromosomes) are joined by RNA splicing. Trans-splicing of exons and introns has been established in the parasite trypanosomes (Borst, 1986) and the flat worm Caenorhabditis elegans (Krause and Hirsh, 1987) and has been suggested for some human genes (Vellard et al., 1992). Clearly, in the case of trans-splicing, both the unit of inheritance and the locus on a chromosome can correspond to a single exon.

It is unlikely, however, that the current working definition of a gene as a linear collection of exons that are joined by RNA splicing will be radically altered in near future. This is probably wise, as the existence of multiple processes collectively called RNA editing further complicates the biochemical definition of a gene (Benne et al., 1986). However, given the possibility that the earliest unit of genetic information may have evolved as an exon, the general concept of exons as gene units may be more valid than any other proposal.

## Splicing of Nuclear Pre-mRNAs

The development of a reaction composed of soluble cellular components that accurately processed pre-mRNAs was critical for advancing our understanding of splicing (Padgett et al., 1983). When combined with the use of highly radioactive pre-mRNA substrates, a biochemical analysis of the splicing process became feasible (Green et al., 1983; Hernandez and Keller, 1983). Not surprisingly, kinetic RNA intermediates in the splicing reaction were soon identified (Grabowski et al., 1984; Krainer et al., 1984). Surprisingly, these intermediates had a lariat structure in which the 5′-most nucleotide of the intron was joined in a 2′–5′ phosphodiester bond to an adenosine within the intron (Konarska et al., 1985). Since the adenosine is covalently bonded through both 3′–5′ and 2′–5′ phosphodiester linkages, this forms an RNA branch. The existence of such branches had just been described from nuclease digestion studies of total nuclear RNA from human cells (Wallace and Edmonds, 1983). Formation of the branch appears during step 1 simultaneously with cleavage at the 5′ splice site and generation of the lariat RNA that typically migrates more slowly than the pre-mRNA during electrophoresis through a tight porosity polyacrylamide gel (Figure 4).

The second step consists of cleavage of the RNA at the 3′ splice site with concomitant joining of the two exons. The intron is released as a lariat RNA and is reasonably stable in reactions in vitro. This contrasts with the situation in vivo in which intron RNAs are almost always rapidly degraded.

The fact that the intermediate state, consisting of two RNAs, was efficiently converted to the final products strongly suggested that these RNAs remain bound in a complex. The complex was identified by its rate of sedimentation in a glycerol gradient, 60S, and was designated to be a spliceosome or splicing body (Grabowski et al., 1985; Brody and Abelson, 1985; Frendewey and Keller, 1985). As anticipated from earlier work suggesting the importance of small nuclear ribonucleoprotein (snRNP) particles in splicing, the spliceosome contained the small nuclear RNAs (snRNAs) U2, U4, U5, and U6 and, under certain conditions, U1 (Steitz et al., 1988). Thus, the spliceosome, much like a ribosome, contains a substrate RNA and a number of stable cellular RNA–protein components.

## Group I, Group II, and the Spliceosome

Comparison of the two steps in splicing by the spliceosome to the RNA-catalyzed self-splicing reactions of group I and II introns shows some striking similarities (Figure 4). In all three cases, the first step is cleavage at the 5′ splice site. In group I introns, this cleavage requires a guanosine that specifically occupies a binding site in the catalytic intron sequences (Cech, 1985). The 3′ hydroxyl group on this guanosine is activated and through a transesterification reaction displaces the 3′ hydroxyl of the 5′ exon. Group II self-splicing introns cleave at the 5′ splice site by activating the 2′-OH at the branch site, producing a lariat RNA (Peebles et al., 1986; van der Veen et al., 1986) much like that produced by the spliceosome. The second step for